CHARGE NUMBER: Project 1902

PROJECT TITLE: Tobacco Microbiology

PERIOD COVERED: October 1-31, 1985

PROJECT LEADER: D. K. Chadick

DATE OF REPORT: October 30, 1985

1. Definition of Spoilage:

Objective: Outline the succession of events during the deterioration of SEL and RL sheet.

Status: A positive control sheet has been created using a low inoculum of our group of four known spoilage bacteria. The four cultures were grown for four hours in a suitable medium and inoculated onto sterile RL/TC sheet in a 1:1:1:1 ratio. The starting level of organisms on the sheet was 1.9×10^5 bacteria/g of sheet. This number is considerably lower than the inoculum level (~1.0×10^8 bacteria/g) used in other positive control experiments. By using a lower amount of organisms, we were able to observe the effect on the sheet in a more realistic manner; i.e. "bad" sheet (based on acetic acid amounts) that we have extracted for organisms and counted seldom has had more than 1.0×10^6-1.0×10^7 bacteria/g of sheet.

The inoculated sheet was stored at 37.5°C and 80% RH for six days. Samples were extracted for bacterial counts and acetic acid analysis at 0 time (just prior to inoculation) and on days 3, 4, 5 and 6. The organism count (2.3×10^7 bacteria/g of sheet) was still increasing at day six. This was in contrast to previous experiments which employed larger amounts of inoculum. In these latter series of experiments peak organism number was achieved in 4-5 days. The acetic acid values for the current experiment are not yet available.

Plans: Repeat this type of positive control sheet experiment using a lower level of inoculum for a longer period of time. At the request of Chris Kroustalis, we also plan to inoculate sheet with the larger number of bacteria, incubating at 37.5°C and 80% RH and withdrawing daily samples for Chris Kroustalis. Chris will use these samples for a variety of analyses and plate counts will also be done.

2. TEG Solvent Replacement in SEL:

Objective: Evaluate suitable solvents to replace TEG in SEL. The selected solvent should be compatible with the propylparaben preservative and act as a humectant in the finished sheet.

Status: Two experiments have been performed using different solvents/humectants with and without paraben in SEL, made without class tobacco in the C Pilot Plant, that had been inoculated with the same group of four known spoilage bacteria (KSB) used in the positive control sheet experiments. The bacteria were used in 1:1:1:1 ratio. The first test

involved the use of propylene glycol(PG), glycerin (G), sorbitol (S), and triethylene glycol (TEG) with and without paraben. The solvent/humectant levels remained constant at 10% (v/v) and 20,50, and 100 ppm propylparaben were used where necessary. Sterile SEL was inoculated with the appropriate amounts of the solvents/humectants and paraben to yield the above mentioned amounts and 10% (v/v) bacterial mixture was also added (final concentration=$9x10^7$ bacteria/ml). Two controls were also included in this study, one flask contained only sterile SEL the other contained sterile SEL with a 10% inoculum of bacterial mixture. The flasks were incubated at 37.5°C and 150 rpm for five days. Samples were removed daily for bacterial plate counts, acetic acid analysis, and propylparaben analysis.

The following results were observed after 5 days of incubation:

1. The solvent controls alone affected the growth of the bacterial mixture compared to the control (bacterial mixture only). PG decreased the bacterial counts in 24h to $2.5x10^4$ organisms/ml. As expected, not much acetic acid was produced in the culture containing only organisms and PG.

2. Sorbitol showed a slight decrease in the bacterial counts at the five day period ($3x10^6$ bacteria/ml) with an increase in acetic acid production (325 ppm) but not to the level as seen in the control which contained the microbes only (at time 0 acetic acid was about 15 ppm and increased to 450 ppm after five days incubation). Glycerin alone caused a decline in the bacterial counts to levels similar to that obtained with PG. However, the culture containing only glycerin required five days to effect this decrease while PG took only one day. Thus, as expected, the glycerin culture showed elevated acetic acid production (375 ppm after five days). Finally the flask with TEG and organisms actually exhibited an increase in bacterial concentration for the five day period ($7x10^8$ organisms/ml versus $1x10^7$ bacteria/ml for the culture without any solvent at the end of five days of incubation). However, the TEG flask showed a decreased level of acetic acid (250 ppm) versus the control (450 ppm). All of the above mentioned results were probably a direct result of the high solvent concentration (10% v/v) employed in these experiments.

3. As expected, for the most part the addition of 20,50, and 100 ppm propylparaben with the solvents/humectants had little effect on the growth of the bacteria after five days. There was some initial decrease in the bacterial counts after 24 hrs with all levels of paraben in all the experiments;however, bacterial recovery occured shortly thereafter. Also, after five days the acetic acid levels were slightly lower than the inoculated SEL control (200-350 ppm). This observation on the effect of propylparaben is not surprising since all current literature stresses that propylparaben offers a bacteristatic and not a bactericidal effect. However, the results with the paraben could be slightly misleading since 10% levels of the solvents/humectants were used. In the next group of experiments these solvent/humectant levels were employed at more realistic amounts.

A second experiment similar in nature to that previously mentioned was conducted using 1.5% TEG, 1.8% PG, and 1.8% PG + 1.5% glycerin with and without propylparaben. The levels of paraben were as follows: 100, 200, 300 and 400 ppm. All solvent/humectant percentages are on a (v/v) basis. The

same type of sterile SEL was used as above and each flask was inoculated with a 10% inoculum of the same known group of four bacteria ($1.9x10^7$ bacteria/ml) in the same ratio as previously mentioned. The flasks were incubated four days at 37.5°C at 150 rpm. The same two controls used in experiment I were also used. After four days of incubation the inoculated control contained $8x10^5$ bacteria/ml. A four day experiment was chosen because it was noticed in the first experiment, while using less paraben, that the effect, if any, was complete by day four.

Bacterial data collection for this experiment is complete and is being tabulated, samples have been submitted for fatty acid analysis, and the results will be reported next month.

The levels of solvents/humectants (with the exception of the first experiment) and propylparaben used throughout both of these groups of experiments were chosen, bearing in mind, the amounts of these compounds that can be realisticaly used at Park 500 production facility (1).

Plans: Repeat this study after all the current data has been analyzed and discussed with the appropriate personnel.

3. Humectant Replacement Trials in C Pilot Plant:

Objective: Store RL/TC and RL/150B sheet that was made with different humectants and propylparaben levels and analyze for changes in the microbial population.

Status: Samples of the acceptable sheet are currently being stored in our environmental rooms at 37.5°C and 25°C with 80% RH maintained at both temperatures. The sheet will be analyzed for bacteria, yeasts, and mold at 1, 2 and 4 week intervals and at 2 and 3 months. Acetic acid analysis will also be done on a monthly basis.

Plans: This is an ongoing study and the data will be reported monthly, based on the storage time of the various sheets.

4. Humectant Replacement Trials at Park 500:

Objective: Store RL/TC and RL/150B sheet that was made with different humectants and propylparaben levels and analyze for changes in the microbial population.

Status: The sheet collection was completed on October 24th, and the samples will be treated as mentioned in item 3. In addition to the storage study done in our environmental rooms, hogsheads of sheet will be stored in a warehouse. At appropriate times, they will be opened and samples will be removed and analyzed as stated in item 3.

Plans: As previously mentioned in item 3.

5. Humectant Replacement Trials at the BL Plant

Objective: Store RL/RCB that was made with different humectants and propyl-paraben levels and analyze for changes in the microbial population.

Status: Sheet has been collected and storage will begin this month as previously described in item 3.

Plans: As previously mentioned in item 4.

6. Mold Analysis of a Silo in OC Containing DBC Bright Tobacco:

Objective: At the request of Ed Craze (OC), samples of DBC bright tobacco in a blend silo in the semi-works were analyzed via plate counts for mold.

Status: A memo was issued and recommendations were made to prevent a future occurrence of the molding problem (2).

7. Bacterial Analysis of an Adhesive With and Without the Addition of Licorice:

Objective: At the request of Sue Wrenn, two samples of a sucrose-Ca acetate binder with and without the addition of 0.75% licorice were analyzed for bacterial contamination. This request was made in an attempt to explain a loss in stability of the adhesive with licorice. A sample of the licorice was also analyzed for bacteria.

Status: A memo has been issued and recommendations were made to prevent future possible contamination of the adhesive by the licorice (3).

References

1. Personal communication with B. Semp.

2. Chadick, D., Mold Analysis of a Silo Containing DBC Bright Tobacco. Memo to J. Nguyen, 1985, 24, October.

3. Chadick, D., Bacterial Analysis of a Sugar Adhesive With and Without the Addition of Licorice. Memo to S. Wrenn, 1985, 14, October.

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